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TITLE OF THE INVENTION (500 characters max)

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Respectfully submitted,

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FIELD OF THE INVENTION

The invention relates generally to the field of molecular biology, biochemistry and agriculture. More particularly, the invention relates to polynucleotides suitable for regulating gene expression in plants and generation of transgenic plants with improved quality and productivity.

BACKGROUND OF THE INVENTION

Modification of a plant characteristic through genetic engineering depends upon the insertion in the plant genome of a polynucleotide construct containing the gene of interest operably linked to a promoter DNA fragment that is functional in the transgenic plant. Within a plant genome, any single gene is, in general, operably linked to a promoter that will determine when and where, within the plant tissues and organs, the gene should be expressed. Therefore if one wants to express a gene of interest in specific tissues or organs within a transgenic plant and in a temporal regulated manner, tissue-preferred promoters must be used. On the other hand, expression in all plant tissues during all plant life cycle could by achieved by using constitutive promoters.

In a number of specific situations the expression of particular genes in particular tissues or organs would confer to the plant a specific phenotype of interest. For example if one wants to improve the nutritional quality of cereal seeds, it will be convenient to express a gene that confers such characteristics using seed-specific promoters, rather than using constitutive promoters that would allow the gene to be expressed in all plant tissues causing in some cases undesirable plant phenotypes. In another example, if one wants to increase the amounts of cellulose in developing plant vascular tissues of a forest tree, one might introduce in the plant genome a xylem- and/or cambium-preferred promoter operably linked to a heterologous gene encoding an enzyme involved in the cellulose metabolism such that more cellulose molecules could be produced in the developing plant xylem. In another example, the desired phenotype could be obtained by inhibiting the expression of a native gene within a specific plant tissue. This could be done by introducing in the plant genome a construct comprised of a tissue-preferred promoter operably linked to a polynucleotide that would inhibit the expression of the endogenous gene either by anti-

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sense hybridization or by RNA silencing (Matzke (ed.) et al. (2000) *Plant Gene Silencing.* Kluwer Academic Publishers).

Thus far, genetically engineering plants to produce useful traits requires the availability of promoters that would allow the genes of interest to be expressed in a tissue- and timing-specific manner. Thus, isolation and characterization of tissue-preferred, particularly cambium/xylem-preferred, promoters that can serve as regulatory regions for expression of heterologous nucleotide sequences of interest in a tissue-preferred manner, is essential for the genetic engineering of plants exhibiting particular traits.

SUMMARY OF THE INVENTION

The present invention relates to regulatory nucleic acid molecules from the genome of *Populus* sp, their compositions, and methods for regulating expression of heterologous nucleotide sequences in a xylem and/or cambium-preferred manner. The regulatory nucleic acid molecules of the present invention correspond to promoter sequences of genes which are preferably expressed in the cambium and/or in the xylem of *Populus* sp. It is hereafter an object of the invention to provide nucleic acid molecules which encode isoforms of sucrose synthase (SuSy), alpha-tubulin (TUB), arabinogalactan protein (ARAB), caffeic acid 3-O-methyltransferase (COMT), cinnamyl alcohol dehydrogenase (CAD), cinnamate 4-hydroxylase (C4H), cinnamoyl CoA reductase (CCR), ferulate-5-hydroxylase (F5H), sinapyl alcohol dehydrogenase (SAD), UDP-D-glucuronate carboxy-lyase (UDP), lipid transfer protein (LTP) and ag-13 (AG13), that are preferably expressed in the cambium and/or in the xylem of *Populus.* Such nucleic acid molecules are referred to throughout the application as "cambium/xylem-preferred promoters". Methods of using the cambium/xylem-preferred promoters disclosed herein, for regulating expression of heterologous nucleotide sequences in cambium and/or xylem-preferred manner in a plant, is provided.

The cambium/xylem-preferred promoters were identified through the analysis of a collection of Expressed Sequence Tags (ESTs) from *Populus* sp, representing apical shoot, bark, cambium, seed, xylem, leaf and root. Based on the expression profile of those ESTs among the different tissues, twelve genes were shown to be highly and preferably expressed

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in the cambium and/or in the xylem of *Populus*. These genes represent isoforms of sucrose synthase (SuSy), alpha-tubulin (TUB), arabinogalactan protein (ARAB), caffeic acid 3-O-methyltransferase (COMT), cinnamyl alcohol dehydrogenase (CAD), cinnamate 4-hydroxylase (C4H), cinnamoyl CoA reductase (CCR), ferulate-5-hydroxylase (F5H), sinapyl alcohol dehydrogenase (SAD), UDP-D-glucuronate carboxy-lyase (UDP), lipid transfer protein (LTP) and ag-13 (AG13), all of which are preferably expressed in the cambium and/or in the xylem of *Populus*.

The cambium/xylem-preferred promoters comprise novel promoter sequences that initiate and control transcription in a cambium and/or xylem-preferred manner. More particularly, transcriptional initiation regions isolated from the cambium/xylem-preferred promoters whose expression is preferred in the cambium and/or xylem, coding for isoforms of sucrose synthase (SuSy), alpha-tubulin (TUB), arabinogalactan protein (ARAB), caffeic acid 3-O-methyltransferase (COMT), cinnamyl alcohol dehydrogenase (CAD), cinnamate 4-hydroxylase (C4H), cinnamoyl CoA reductase (CCR), ferulate-5-hydroxylase (F5H), sinapyl alcohol dehydrogenase (SAD), UDP-D-glucuronate carboxy-lyase (UDP), lipid transfer protein (LTP) and ag-13 (AG13), are provided. Further compositions of the invention comprise the nucleotide sequence set forth in SEQ ID NOS.: 1-12 and a fragment of the nucleotide sequences set forth in SEQ ID NOS.: 1-12 comprising at least 20 nucleotides. The compositions of the invention further comprise nucleotide sequences having at least 65% identity to the sequences set forth in SEQ ID NOS.: 1-12 or a fragment thereof, and nucleotide sequences that hybridized under high stringency conditions to any one of the above mentioned sequences.

"Stringent conditions" as used herein, refers to parameters with which the art is familiar, such as hybridization in 3.5xSSC, 1xDenhardt's solution, 25mM sodium phosphate buffer (pH 7.0), 0.5% SDS, and 2mM EDTA for 18 hours at 65°C, followed by 4 washes of the filter at 65°C for 20 minutes, in 2xSSC, 0.1% SDS, and a final wash for up to 20 minutes in 0.5xSSC, 0.1% SDS, or 0.3xSSC and 0.1% SDS for greater stringency, and 0.1xSSC, 0.1% SDS for even greater stringency. Other conditions may be substituted, as long as the degree of stringency in equal to that provided herein, using a 0.5xSSC final wash.

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Compositions of the present invention also include constructs comprising the promoters, operably linked to a nucleotide sequence of interest. The promoters disclosed herein are capable of driving expression of polynucleotides of interest in a plant cell and said promoters comprises the nucleotide sequences of the present invention. The invention further provide expression vectors comprising the above mentioned constructs.

Compositions also include plants or plant cells having stably incorporated into their genomes of any one of the constructs described above, which comprises a promoter operably linked to a nucleotide sequence, wherein said promoter is capable of driving expression of said nucleotide sequence in a plant cell and said promoter comprises a nucleotide sequence of the present invention. Additionally, compositions include the seeds of such plants.

Methods of the invention comprise means to introduce, stable incorporation and expression of nucleotide sequences in a plant, said method comprising a construct comprising a promoter operably linked to a nucleotide sequence, wherein said promoter is capable of initiating transcription of said nucleotide sequence in said plant and said promoter comprises a nucleotide sequence of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** schematically illustrates the plasmid vector pAPROM-ATG+promoter comprising the GUS reporter gene operably linked to nucleotide sequence representing a promoter. Promoters of the invention were cloned in this plasmid vector in substitution of the represented promoter sequence.

FIG. **2** schematically illustrates the plasmid vector pAPROM-ATG+SUSY-x comprising the GUS reporter gene operably linked to the SUSY promoter of the invention.

FIG. **3** schematically illustrates the plasmid vector pAPROM-ATG+TUB-x comprising the GUS reporter gene operably linked to the TUB promoter of the invention.

FIG. **4** schematically illustrates the plasmid vector pAPROM-ATG+ARAB-x comprising the GUS reporter gene operably linked to the ARAB promoter of the invention.

FIG. **5** schematically illustrates the plasmid vector pAPROM-ATG+COMT-x comprising the GUS reporter gene operably linked to the COMT promoter of the invention.

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FIG. **6** schematically illustrates the plasmid vector pAPROM-ATG+CAD-x comprising the GUS reporter gene operably linked to the CAD promoter of the invention.

FIG. **7** schematically illustrates the plasmid vector pAPROM-ATG+C4H-x comprising the GUS reporter gene operably linked to the C4H promoter of the invention.

FIG. **8** schematically illustrates the plasmid vector pAPROM-ATG+CCR-x comprising the GUS reporter gene operably linked to the CCR promoter of the invention.

FIG. **9** schematically illustrates the plasmid vector pAPROM-ATG+F5H-x comprising the GUS reporter gene operably linked to the F5H promoter of the invention.

FIG. **10** schematically illustrates the plasmid vector pAPROM-ATG+SAD-x comprising the GUS reporter gene operably linked to the SAD promoter of the invention.

FIG. **11** schematically illustrates the plasmid vector pAPROM-ATG+UDP-x comprising the GUS reporter gene operably linked to the UDP promoter of the invention.

FIG. **12** schematically illustrates the plasmid vector pAPROM-ATG+LTP-x comprising the GUS reporter gene operably linked to the LTP promoter of the invention.

FIG. 13 schematically illustrates the plasmid vector pAPROM-ATG+AG13-x comprising the GUS reporter gene operably linked to the AG13 promoter of the invention.

FIG. 14 shows the expression profile in a set of *Populus* tissues of SuSy, TUB, ARAB, UDP, LTP and AG13, whose promoters are disclosed herein.

FIG. 15 shows the expression profile in a set of *Populus* tissues of COMT, CAD, C4H, CCR, F5H and SAD, whose promoters are disclosed herein.

FIG. 16 schematically illustrates the plasmid vector pALELLYXgi comprising a gene of interest operably linked to one of the cambium/xylem-preferred promoters disclosed herein.

DETAILED DESCRIPTION OF THE INVENTION

The compositions of the present invention comprise novel nucleotide sequences for plant promoters, particularly cambium/xylem-preferred promoters for the *Populus* (woody aspen) genes encoding sucrose synthase (SuSy), alpha-tubulin (TUB), arabinogalactan protein (ARAB), caffeic acid 3-O-methyltransferase (COMT), cinnamyl alcohol dehydrogenase (CAD), cinnamate 4-hydroxylase (C4H), cinnamoyl CoA reductase (CCR),

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ferulate-5-hydroxylase (F5H), sinapyl alcohol dehydrogenase (SAD), UDP-D-glucuronate carboxy-lyase (UDP), lipid transfer protein (LTP) and ag-13 (AG13). The nucleotide sequences for these promoters are set forth in SEQ ID NOS.: 1-12, respectively. These promoters were isolated from the 5′untranslated region flanking the transcription initiation sites of their respective genes. Methods for the isolation of the promoters are well known in the art and include bioinformatic tools for gene assembling such as Phred, Phrap, Consed (Gordon *et al.* (1998) *Genome Research.* 8:195-202), sequence alignment (Durbin *et al.* (1998) *Biological sequence analysis – probabilistic models of proteins and nucleic acids.* Cambridge University Press, Cambridge, UK), functional search (Altschul *et al.* (1997) *Nucleic Acid Res.* 25:3389-3402) and PCR techniques (Sambrook and Russell (2001) *Molecular Cloning – a laboratory manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA). Some of these methods are described in Example 1 below.

The invention encompasses isolated nucleic acid molecules corresponding to the 5′ ends that naturally flank the gene coding regions of nucleic acid sequences from the genomic DNA of *Populus* sp. In various embodiments, the isolated nucleic acid molecules span 0.1 kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5 kb starting at trinucleotide ATG that encodes the first amino acid of a protein or peptide encoded by the gene coding region. The isolated nucleic acid molecules are referred to as promoters. Promoters correspond to the nucleic acid molecules whose function is to regulate the expression of a gene. A promoter generally comprises specific signaling sequences called boxes, arranged along the promoter sequence, so that its composition determines the temporal and spatial expression of a gene that is under its regulatory control. By "promoter" or "transcriptional initiation region" is intended to mean a regulatory region of DNA usually comprising a TATA box capable of directing RNA polymerase II to initiate RNA synthesis at the appropriate transcription initiation site for a particular coding sequence. A promoter may additionally comprise other recognition sequences generally positioned upstream or 5′ to the TATA box, referred to as upstream promoter elements, which influence the transcription initiation rate. It is recognized that having identified the nucleotide sequences for the promoter regions disclosed herein, it is within the state of the art to isolate and identify further regulatory

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elements in the 5′ untranslated region upstream from the particular promoter regions identified herein.

Thus the promoter regions disclosed herein are generally further defined by comprising upstream regulatory elements such as, those responsible for tissue and temporal expression of the coding sequence, enhancers and the like. In the same manner, the promoter elements, which enable expression in the desired tissue such as the xylem and/or cambium, can be identified isolated and used with other core promoters to confer cambium/xylem-preferred expression.

In the present invention, a series of promoters that regulate the expression of genes specifically in the cambium and/or xylem, were identified and isolated from *Populus* sp.

The Susy gene encodes an isoform of sucrose synthase, an enzyme involved in the enzymatic conversion of sucrose into UDP-glucose in the developing xylem. UDP-glucose is the building block of cellulose that is synthesized and deposited in the cell-wall of plant cells. The Susy gene disclosed herein is preferentially expressed in the cambium/xylem of *Populus* sp, although low levels of expression can be observed in other tissues (FIG. 14).

The TUB gene encodes an isoform of alpha-tubulin, a structural globular protein involved in the formation of microtubules, which are themselves part of the cytoskeleton. The TUB gene disclosed herein is preferentially expressed in the cambium and/or xylem of *Populus* sp as, although low levels of expression can be observed in other tissues (FIG. 14).

The ARAB gene encodes an isoform of arabinogalactan protein, member of a large family of plant cell wall-associated glycoproteins of unknown function. The ARAB gene disclosed herein is preferentially expressed in the cambium/xylem of *Populus* sp, although low levels of expression can be observed in other tissues (FIG. 14).

The COMT gene encodes an isoform of caffeic acid 3-O-methyltransferase implicated in the methylation of both caffeic acid and 5-hydroxyferulic acid. These are intermediary compounds of lignin biosynthesis. The COMT gene disclosed herein is preferentially expressed in the cambium/xylem of *Populus* sp, although low levels of expression can be observed in other tissues (FIG. 15).

The CAD gene encodes an isoform of cinnamyl alcohol dehydrogenase, an enzyme that catalyzes the final step in the synthesis of the monolignols, thereby converting the

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cinnamaldehydes to the corresponding alcohols. The CAD gene disclosed herein is preferentially expressed in the cambium/xylem of *Populus* sp, although low levels of expression can be observed in other tissues (FIG. 15).

The C4H gene encodes an isoform of cinnamate 4-hydroxylase, a member of the cytochrome P450 monooxygenase superfamily involved in the catalysis of the first oxidative reaction in phenylpropanoid metabolism, the conversion of trans-cinnamic acid to *p*-coumaric acid. The C4H gene disclosed herein is preferentially expressed in the cambium/xylem of *Populus* sp, although low levels of expression can be observed in other tissues (FIG. 15).

The CCR gene encodes an isoform of the cinnamoyl CoA reductase, which catalyzes the conversion of cinnamoyl CoA esters to their corresponding cinnamaldehydes, i.e., the first specific step in the synthesis of the lignin monomers. The CCR gene disclosed herein is preferentially expressed in the cambium/xylem of *Populus* sp, although low levels of expression can be observed in other tissues (FIG. 15).

The F5H gene encodes a cytochrome P450-dependent monooxygenase that catalyzes the hydroxylation of ferulic acid toward sinapic acid and syringyl lignin biosynthesis. The F5H gene disclosed herein is preferentially expressed in the cambium/xylem of *Populus* sp, although low levels of expression can be observed in other tissues (FIG. 15).

The SAD gene encodes a sinapyl alcohol dehydrogenase that mediates the reduction of sinapaldehyde into syringyl monolignols in angiosperms. The SAD gene disclosed herein is preferentially expressed in the cambium/xylem of *Populus* sp, although low levels of expression can be observed in other tissues (FIG. 15).

The UDP gene encodes the enzyme UDP-D-glucuronate carboxy-lyase involved in the breakdown of UDP-D-glucuronate into UDP-D-xylose and CO_2. The UDP gene disclosed herein is preferentially expressed in the cambium/xylem of *Populus* sp, although low levels of expression can be observed in other tissues (FIG. 14).

The LTP gene encodes an isoform of lipid transfer protein, a member of a family thought to participate in cutin formation, embryogenesis, defense reactions against phytopathogens, symbiosis, and the adaptation of plants to various environmental

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conditions. The LTP gene disclosed herein is preferentially expressed in the cambium/ xylem of *Populus* sp, although low levels of expression can be observed in other tissues (FIG. 14).

The AG13 gene encodes an ag-13 protein of unknown function, whose expression has been associated with the ripening process in several plant species. The AG13 gene disclosed herein is preferentially expressed in the cambium/xylem of *Populus* sp, although low levels of expression can be observed in other tissues (FIG. 14).

The cambium/xylem-preferred promoter sequences of the present invention drive the expression of operably linked nucleotide sequences in a cambium/xylem-preferred manner. The EXAMPLE 3 illustrates the expression of the GUS reporter gene in the cambium/xylem vessels complex of *Arabidopsis thaliana* transformed with a construct containing the GUS reporter gene operably linked to the cambium/xylem-preferred promoters of the invention, TUB (SEQ ID.: 2) and C4H (SEQ ID.: 6). Thus, the cambium/xylem-preferred promoter sequences disclosed herein can be used to express an operably linked sequence of interest in the cambium and/or in the xylem. Hence, the cambium/xylem-preferred promoters can be used to improve the wood quality of wood trees either by increasing the synthesis of cellulose or decreasing the synthesis of lignin. By "decreasing lignin synthesis" is intended to mean decreasing the total lignin content of woody trees by 1%, 5%, 10%, 15%, 20%, 30%, 50%, 70% and most preferably 80% and 90% compared with normal field grown plants. By "increasing cellulose synthesis" is intended to mean increasing the total cellulose content of woody trees by 1%, 5%, 10%, 15%, 20%, 30%, 50%, 70% and most preferably 80% and 90% compared with normal field grown plants.

In addition, the cambium/xylem-preferred promoters can be used to inhibit the expression of genes involved in the developing xylem metabolism. The inhibition of such genes would decrease the concentration of lignin and/or change the relationship between guaiacyl and syringyl, the building blocks of lignins. The monomeric composition of lignins is one of the important characteristics from an industrial point of view, because syringyl unit-rich lignins are more easily degraded during the pulping process since they contain fewer strong 5-5' carbon bonds. Thus, the determination of the syringyl to guaiacyl

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(S/G) ratio is useful to evaluate wood quality for paper production (Boudet et al., 1998). By "changing the relationship between syringyl and guaiacyl" is intended to mean increasing the syringyl/guaiacyl ratio by 1%, 5%, 10%, 15%, 20%, 30%, 50%, 70% and most preferably 80% and 90% compared with normal field grown plants.

Other nucleic acid molecules within the invention are variants and/or fragments of the cambium/xylem-preferred promoter sequences such as those that encode fragments, analogs and derivatives of native cambium/xylem-preferred promoter sequences disclosed herein. Such variants and/or fragments may be, e.g., naturally occurring variants of native cambium/xylem-preferred promoter sequences, or a non-naturally occurring variant of cambium/xylem-preferred promoter sequences. For example, the nucleotide sequence of such variants and/or fragments can feature a deletion, addition, or substitution of one or more nucleotides of native cambium/xylem-preferred promoter sequences. Such variants and/or fragments may retain the biological activity and therefore drive, in a cambium/xylem-preferred manner, the expression of operably linked nucleotide sequences. Fragments of cambium/xylem-preferred promoter sequences comprise at least 10, 20, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1500, 2000, 3000, 4000 nucleotides or up to the number of nucleotides in the full-length cambium/xylem-preferred promoter sequences disclosed herein as for example the 700-3500 nucleotides for SEQ ID NOS.:1-12.

By "variants" is intended to mean substantially similar sequences. Naturally and non-naturally occurring "variants" of cambium/xylem-preferred promoter sequences within the invention are nucleic acids having at least 65% sequence identity with the native cambium/xylem-preferred promoter sequences disclosed herein. "Variants" also include nucleic acids molecules that hybridize under stringent conditions as defined herein to the cambium/xylem-preferred promoter nucleic acid sequences such as that of SEQ ID NOS.: 1-12 or the complement of the sequences of SEQ ID NOS.: 1-12. For example, such "variants" may be nucleic acids that hybridize to the sequence of SEQ ID NOS.: 1-12 or the complement of the sequences of SEQ ID NOS: 1-12 under low stringency conditions, moderate stringency conditions, or high stringency conditions, are within the invention. Preferred, such nucleic acids are those having a nucleotide sequence that is the complement

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of the full-length or portions of the sequences of SEQ ID NOS.: 1-12. Other variants of cambium/xylem-preferred promoter sequences within the invention are polynucleotides that share at least 65% sequence identity, preferably at least 80%, more preferably at least 90%, and most preferably at least 95%, to the sequences of SEQ ID NOS: 1-12 or the complement of the sequences of SEQ ID NOS: 1-12.

"Stringent conditions" as used herein, refers to parameters with which the art is familiar, such as hybridization in 3.5xSSC, 1xDenhardt's solution, 25mM sodium phosphate buffer (pH 7.0), 0.5% SDS, and 2mM EDTA for 18 hours at 65°C, followed by 4 washes of the filter at 65°C for 20 minutes, in 2xSSC, 0.1% SDS, and a final wash for up to 20 minutes in 0.5xSSC, 0.1% SDS, or 0.3xSSC and 0.1% SDS for greater stringency, and 0.1xSSC, 0.1% SDS for even greater stringency. Other conditions may be substituted, as long as the degree of stringency in equal to that provided herein, using a 0.5xSSC final wash.

For purposes of the present invention, sequence identity to any of the promoter sequences disclosed herein is preferably made using the BLAST program or any sequences alignment program that allows the alignment of identical nucleotides and verification of mismatches between non-identical nucleotides so that the percentage of identity of compared sequences could be estimated.

The cambium/xylem-preferred promoters of the invention may be used to express a gene of interest. For example, by using cambium/xylem-preferred promoters, the expression of native and/or non-native genes could be regulated in the cambium and/or xylem tissues of a plant, altering a plant's cellulose content, lignin content, pathogen or insect resistance, wood development, wood quality, and the like. The native and/or non-native genes includes those encoding enzymes, transporters, cofactors, transcription factors and a number of other genes that would affect cellulose and/or lignin deposition in the plant or pathogen or insect resistance.

For the present invention, "genes of interest" include those involved in cellulose metabolism, such as cellulose synthase and sucrose synthase, and those involved in lignin metabolism, such as coniferaldehyde-5-hydroxylase (Cald5H) and 4-coumarate-CoA-ligase

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(4CL). It is recognized that any gene of interest can be operably linked to the promoter of the invention and expressed in plant cambium and/or xylem tissues.

The cambium/xylem-preferred promoters of the present invention, when operably linked to a gene of interest and stably incorporated into a plant genome drive cambium and/or xylem-preferred expression of the said gene of interest. The cambium and/or xylem-preferred expression is intended to mean that expression of the gene of interest is most abundant in the cambium and/or in the xylem although some level of expression of the gene of interest may occur in other plant tissues. By cambium is intended to mean any part of the cambial or procambial tissue in any organ of the plant, including but not limited to the root, shoot, stem, wood, leaf, petiole, and the like. By xylem is intended to mean any part of the xylem tissue, including but not limited to the tracheids, tracheary elements, vessels, fuse fibers and pith. Some of the promoters disclosed herein may drive the expression of genes to the secondary xylem more prominently than to the primary xylem.

The constructs containing the cambium/xylem-preferred promoters disclosed in the present invention, and an operably linked gene of interest may be provided in expression cassettes as depicted in FIGs. 2-13. Such expression cassettes comprise the cambium/xylem-preferred promoters of the present invention, or variants or fragments thereof, operably linked to a gene of interest whose expression is directed to the cambium and/or xylem. Such an expression cassette may contain restriction sites for insertion of the gene of interest under the transcriptional control of the cambium/xylem-preferred promoters. The expression cassette may additionally contain a number of other nucleic acid sequences, including selectable marker genes, transcriptional and translational initiation sequences, and a plant transcriptional and translational termination sequence. The termination region may be native with the DNA sequence of interest, may be from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions (REF). Reporter genes or selectable marker genes may be included in the expression cassettes. Examples of suitable reporter genes known in the art can be found in, for example, Jefferson et al. (1991) in *Plant Molecular Biology Manual*, ed. Gelvin et al. (Kluwer Academic Publishers), pp. 1-33. Selectable marker genes for selection of transformed cells or tissues can include genes that confer herbicide resistance. Examples of

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suitable selectable marker genes include, but are not limited to, genes encoding resistance to sulfonamide (Guerineau et al. (1990) *Plant Mol. Biol.* 15:127-136); bromoxynil (Stalker et al. (1988) *Science* 242:419-423); glyphosate (Shaw et al. (1986) *Science* 233:478-481); phosphinothricin (DeBlock et al. (1987) *EMBO J.* 6:2513-2518). The expression cassettes of the present invention operably linked to a gene of interest are useful for the transformation of a variety of plants. Such plants, include, but are not limited to, *Eucalyptus* species (*E. alba, E. albens, E. amygdalina, E. aromaphloia, E. baileyana, E. balladoniensis, E. bicostata, E. botryoides, E. brachyandra, E. brassiana, E. brevistylis, E. brockwayi, E. camaldulensis, E. ceracea, E. cloeziana, E. coccifera, E. cordata, E. cornuta, E. corticosa, E. crebra, E. croajingolensis, E. curtisii, E. dalrympleana, E. deglupta, E. delegatensis, E. delicata, E. diversicolor, E. diversifolia, E. dives, E. dolichocarpa, E. dundasii, E. dunnii, E. elata, E. erythrocorys, E. erythrophloia, E. eudesmoides, E. falcata, E. gamophylla, E. glaucina, E. globulus, E. globulus subsp. bicostata, E. globulus subsp. globulus, E. gongylocarpa, E. grandis, E. grandis x urophylla, E. guilfoylei, E. gunnii, E. hallii, E. houseana, E. jacksonii, E. lansdowneana, E. latisinensis, E. leucophloia, E. leucoxylon, E. lockyeri, E. lucasii, E. maidenii, E. marginata, E. megacarpa, E. melliodora, E. michaeliana, E. microcorys, E. microtheca, E. muelleriana, E. nitens, E. nitida, E. obliqua, E. obtusiflora, E. occidentalis, E. optima, E. ovata, E. pachyphylla, E. pauciflora, E. pellita, E. perriniana, E. petiolaris, E. pilularis, E. piperita, E. platyphylla, E. polyanthemos, E. populnea, E. preissiana, E. pseudoglobulus, E. pulchella, E. radiata, E. radiata subsp. radiata, E. regnans, E. risdonii, E. robertsonii, E. rodwayi, E. rubida, E. rubiginosa, E. saligna, E. salmonophloia, E. scoparia, E. sieberi, E. spathulata, E. staeri, E. stoatei, E. tenuipes, E. tenuiramis, E. tereticornis, E. tetragona, E. tetrodonta, E. tindaliae, E. torquata, E. umbra, E. urophylla, E. vernicosa, E. viminalis, E. wandoo, E. wetarensis, E. willisii, E. willisii subsp. falciformis, E. willisii subsp. willisii, E. woodwardii*), *Populus* species (*P. alba, P. alba x P. grandidentata, P. alba x P. tremula, P. alba x P. tremula var. glandulosa, P. alba x P. tremuloides, P. balsamifera, P. balsamifera subsp. trichocarpa, P. balsamifera subsp. trichocarpa x P. deltoides, P. ciliata, P. deltoides, P. euphratica, P. euramericana, P. kitakamiensis, P. lasiocarpa, P. laurifolia, P. maximowiczii, P. maximowiczii x P. balsamifera subsp. trichocarpa, P. nigra, P. sieboldii x*

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P. grandidentata, P. suaveolens, P. szechuanica, P. tomentosa, P. tremula, P. tremula x P. tremuloides, P. tremuloides, P. wilsonii, P. canadensis, P. yunnanensis) and Conifers as, for example, loblolly pine (*Pinus taeda*), slash pine (*Pinus elliotii*), ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), and Monterey pine (*Pinus radiata*); Douglas-fir (*Pseudotsuga menziesii*); Western hemlock (*Tsuga canadensis*); Sitka spruce (*Picea glauca*); redwood (*Sequoia sempervirens*); true firs such as silver fir (*Abies amabilis*) and balsam fir (*Abies balsamea*); and cedars such as Western red cedar (*Thuja plicata*) and Alaska yellow-cedar (*Chamaecyparis nootkatensis*).

The expression cassettes may be stably incorporated into plant genomes by *Agrobacterium*-mediated transformation (Fraley *et al.* (1983) *Proc. Natl. Acad. Sci. USA.* 80:4803-4807) and biobalistic (Klein *et al.* (1987) *Nature*. 327:70-73).

All technical terms used herein are terms commonly used in biochemistry, molecular biology and agriculture, and can be understood by one of ordinary skill in the art to which this invention belongs. Those technical terms can be found in: Molecular Cloning: A Laboratory Manual, 3rd ed., vol. 1-3, ed. Sambrook and Russel, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; Current Protocols in Molecular Biology, ed. Ausubel et al., Greene Publishing Associates and Wiley-Interscience, New York, 1988 (with periodic updates); Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, 5th ed., vol. 1-2, ed. Ausubel et al., John Wiley & Sons, Inc., 2002; Genome Analysis: A Laboratory Manual, vol. 1-2, ed. Green et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1997. Methods involving plant biology techniques are described herein and are described in detail in methodology treatises such as Methods in Plant Molecular Biology: A Laboratory Course Manual, ed. Maliga et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1995. Various techniques using PCR are described, e.g., in Innis et al., PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego, 1990 and in Dieffenbach and Dveksler, PCR Primer: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2003. PCR-primer pairs can be derived from known sequences by known techniques such as using computer programs intended for that purpose (e.g., Primer, Version 0.5, 1991, Whitehead Institute for Biomedical Research,

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Cambridge, MA). Methods for chemical synthesis of nucleic acids are discussed, for example, in Beaucage and Caruthers (1981) Tetra. Letts. 22:1859-1862 and Matteucci and Caruthers (1981) J. Am. Chem. Soc. 103:3185.

The present invention is further illustrated by the following specific examples. The examples are provided for illustration only and are not to be construed as limiting the scope or content of the invention in any way.

EXPERIMENTAL

The promoter region for the genes coding for sucrose synthase (SuSy), alpha-tubulin (TUB), arabinogalactan protein (ARAB), caffeic acid 3-O-methyltransferase (COMT), cinnamyl alcohol dehydrogenase (CAD), cinnamate 4-hydroxylase (C4H), cinnamoyl CoA reductase (CCR), ferulate-5-hydroxylase (F5H), sinapyl alcohol dehydrogenase (SAD), UDP-D-glucuronate carboxy-lyase (UDP), lipid transfer protein (LTP) and ag-13 (AG13) were isolated from *Populus deltoides* (aspen, poplar). The nucleotide sequences for these promoters are set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, respectively. The method for their isolation is described below.

EXAMPLE 1

Expression Profile of Genes Preferred-Expressed in Cambium/Xylem

Expressed Sequence Tags (ESTs) from *Populus* sp. were clustered using the CAP3 program (Huang and Madan (1999) *Genome Res.* 9:868-877). The ESTs were obtained from libraries representing the following tissues: apical shoot, bark, cambium, seed, xylem, leaf and root. The set of clusters thus generated was searched for clusters composed of at least 90% of ESTs from libraries representing *Populus* cambium and xylem tissues. Twelve clusters were chosen among those based on their high and preferred level of expression in the cambium and/or in the xylem of *Populus*. A BLASTX search against the non-redundant GenBank database was then performed with each one of the twelve clusters, and it was concluded that these represent expressed sequences from the following genes, whose

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promoters are disclosed herein: sucrose synthase (SuSy), alpha-tubulin (TUB), arabinogalactan protein (ARAB), caffeic acid 3-O-methyltransferase (COMT), cinnamyl alcohol dehydrogenase (CAD), cinnamate 4-hydroxylase (C4H), cinnamoyl CoA reductase (CCR), ferulate-5-hydroxylase (F5H), sinapyl alcohol dehydrogenase (SAD), UDP-D-glucuronate carboxy-lyase (UDP), lipid transfer protein (LTP) and ag-13 (AG13). Figures 1 and 2 shows the expression profile in several tissues of *Populus* for each of the clusters representing the genes whose promoters are disclosed herein. The series of histograms in FIG 14 and FIG 15 depict the relative abundance of the mRNA in each tissue used for the construction of cDNA libraries representing the aforementioned tissues (apical shoot, bark, cambium, seed, xylem, leaf and root). The histograms compose a set of digital expression data which is an approximation of the relative level of expression for the twelve genes whose promoters are disclosed herein.

EXAMPLE 2

Isolation of Promoter Sequences

BLASTN was performed for each one of the twelve clusters against the genomic sequences from *Populus trichocarpa* made available by the Joint Genome Institute, US Department of Energy as part of the "Populus Genome Sequencing Project" (http://genome.jgi-psf.org/poplar0/poplar0.info.html). Selected nucleotide regions from each cluster corresponding to putative exons were used as driver sequences in the retrieval of genomic sequence reads comprising the transcription initiation region and adjacent upstream promoter sequences. These genomics reads were assembled using the PHRAP (Gordon *et al.* (1998) *Genome Res.* 8:195-202) program to obtain a contig encompassing approximately 700 to 3500 nucleotides of putative promoter region upstream of the transcription initiation point (+1 nucleotide, which corresponds to the beginning of the respective mRNA). These contigs contain the promoter regions for each of the genes encoding the mRNAs represented by the twelve clusters concluded to be preferably expressed in the cambium and/or in the xylem tissues of *Populus*. These twelve promoter regions correspond to sequences disclosed herein are set forth in SEQ ID NOS.: 1-12.

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For isolation of specific promoter regions, two anti-parallel gene-specific primers (usually 30 nt in length) were designed from the sequences of the promoter contigs described above to amplify by PCR a fragment of approximately 900 to 1500 nucleotides from the promoter region of each one of the twelve genes whose promoter sequences are disclosed herein. The first round of PCR was performed on genomic DNA sample from *Populus deltoides* or *P. trichocharpa*, which was prepared from leaves using the cetyltrimethyl-ammonium bromide (CTAB) extraction method (Aldrich and Cullis (1993) *Plant Mol. Biol. Report.* 11:128-141). The primers were designed to amplify the region upstream of the coding sequence, i.e., the 5' untranslated region and promoter region of the chosen gene. The sequences of the primers are given below for each promoter described:

sucrose synthase (SuSy)

5′- GCCATAGCTCCTTAAGAGAAACAGAAAGCAA -3′	(SEQ ID NO: 13)
5′- CAATATAGAATCAATGAACAGCACTAGTTTGC -3′	(SEQ ID NO: 14)
5′- TCATGTCCTATCCAACGGCG - 3′	(SEQ ID NO: 15)

alpha-tubulin (TUB)

5′- CTCATTTTCTCTCAAAGCTCAAAG -3′	(SEQ ID NO: 16)
5′- GACAACTAGTCTAAAGTTAAAACTTAGACC -3′	(SEQ ID NO: 17)
5′- CCCTGGAGGTTGGGGTGAGT - 3′	(SEQ ID NO: 18)

arabinogalactan protein (ARAB)

5′- GCGTTCATCTACAAAACCCTCCTCC -3′	(SEQ ID NO: 18)
5′- TTCATCCTTATTTTTTTGGGATA -3′	(SEQ ID NO: 19)
5′- CAAAGGATCATGGAGTTGGA - 3′	(SEQ ID NO: 20)

caffeic acid 3-O-methyltransferase (COMT)

5′- TATACTAATATGACCTAATAACTTAGAAGTGTGG -3′	(SEQ ID NO: 21)
5′- CATCTTGATCAAGATTGAATTC -3′	(SEQ ID NO: 22)
5′- CATAATATCAAAACTTAAGC - 3′	(SEQ ID NO: 23)

cinnamyl alcohol dehydrogenase (CAD)

5′- TGAATTGATGACGTAGGAAACATGATAAACATG -3′	(SEQ ID NO: 24)
5′- CATTTTCTTGAAACAATGAGGCTAAGAG -3′	(SEQ ID NO: 25)

cinnamate 4-hydroxylase (C4H)

5′- GACATGAGAAACTAACGTTGCTTGAATTC -3′	(SEQ ID NO: 26)
5′- CATAATATTGGAACTGGTTTCTTTGTCAGAAAG -3′	(SEQ ID NO: 27)

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cinnamoyl CoA reductase (CCR)

5′- GCGCTCGGGTTGTCACCATAGTTTC -3′	(SEQ ID NO: 28)
5′- CATGTTGTTATATTTAGATAAATGTA -3′	(SEQ ID NO: 29)

ferulate-5-hydroxylase (F5H)

5′- TTCATCAAGCAATAATAATAAGGTGAGGC -3′	(SEQ ID NO: 30)
5′- CATGGATGCAGATTTTTGTGTTTGTG -3′	(SEQ ID NO: 31)
5′- AAAGGCATCG ATTATAGATG - 3′	(SEQ ID NO: 32)

sinapyl alcohol dehydrogenase (SAD)

5′- AATCGAAACCGATCGATTTGAACTGG -3′	(SEQ ID NO: 33)
5′- CATGGTGCTTGCTTCAGATAG -3′	(SEQ ID NO: 34)

UDP-D-glucuronate carboxy-lyase (UDP)

5′- GGAAATGTCAACACTTGTGTGACCACAC -3′	(SEQ ID NO: 35)
5′- GACATTCTTGTCCAATTTCTGAA -3′	(SEQ ID NO: 36)

lipid transfer protein (LTP)

5′- GGAGCCTCCATATTTCTGTATCTC -3′	(SEQ ID NO: 37)
5′- CAAGACGATGAAATGAAGAACTGATAGC -3′	(SEQ ID NO: 38)

ag-13 (AG13)

5′- GACATTCCTTGACTTAATATGATGTTGATAGTC -3′	(SEQ ID NO: 39)
5′- ACAGTGGCCATTGGAACTAGAAGG -3′	(SEQ ID NO: 40)

PCR was performed on a model 9700 GeneAmp thermal cycler (Applied Biosystems) using reagents supplied with the AmpliTaq DNA polymerase (Applied Biosystems). The following cycle parameters were generally used: 5 min at 94° C followed by 35 cycles of 94° C for 1 min, then a varying annealing temperature, as described infra for 1 min, then 72° C for 3 min. The annealing temperature (T) was adjusted for each primer pair and ranged from 50° C to 59° C. Finally, the samples were held at 72° C for 4 min, then at 4° C until further analysis. Ten µl of each of the resulting amplified DNA fragments was run out on a 0.8% agarose gel, purified using the GFX Gel Purification Kit (Amersham), subcloned into pGEM-T-Easy vector (Promega) and then into EcoRI and BglII sites of the pAPROM-ATG vector. Final sequences were determined on the resulting plasmids. Figure 3 schematically illustrates the expression cassette pAPROM-ATG comprising the GUS gene operably linked to a promoter disclosed herein. Figure 4

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schematically illustrates the plasmid vector comprising a gene of interest operably linked to a promoter of the invention.

EXAMPLE 3

Transformation of Arabidopsis Plants

Arabidopsis thaliana Columbia plants are transformed using an *Agrobacterium tumefaciens* mediated transformation protocol (Bechtold *et al.*, (1993) *C. R. Acad. Sci. Paris* 316:1194-1199; Bent *et al.*, (1986) *Mol. Gen. Genet.* 204:383-396) with individual constructs containing any one of the promoters of the invention (SuSy, TUB, ARAB, COMT, CAD, C4H, CCR, F5H, SAD, UDP, LTP or AG13) operably linked to a gene of interest. The constructs also contain the selectable marker gene *Bar* that confers resistance to herbicidal phosphinothricin analogs like ammonium gluphosinate (Thompson *et al.* (1987) *EMBO J.* 9:2519-2523). In this example the gene of interest operably linked to the cambium/xylem-preferred promoters of the invention is the reporter gene *Gus* encoding the enzyme beta-glucuronidase (GUS) (Jefferson (1987) *Plant Mol. Biol. Rep.* 5: 387-405) that facilitates visual inspection of the desirable phenotype, i.e., expression of GUS in a cambium/xylem-preferred manner.

Seeds of *Arabidopsis thaliana* ecotype Columbia are sown in pots containing vermiculite. Plants are grown at 16/8 hours dark/light regime at 22 °C. After 4-5 weeks plants are transformed with the *Agrobacterium tumefaciens* strain GV3101 (C58C1 rifampicin) pMP90 (gentamicin) (Bent *et al.*, (1986) *Mol. Gen. Genet.* 204:383-396) harboring the plasmid vector comprising the gene of interest operably linked to any one of the promoters SuSy, TUB, ARAB, COMT, CAD, C4H, CCR, F5H, SAD, UDP, LTP and AG13 of the invention (SEQ ID NOs.: 1-12).

For plant transformation, 1 litter of LB medium containing rifampicin, gentamycin and kanamicin is inoculated with an aliquot of overnight starter *Agrobacterium* culture. The culture is grown overnight at 28 °C in a rotatory shaker, until OD600 is $\geq$ 8.0. The *Agrobacterium* is precipitated by centrifugation and the bacteria pellet is resuspended in ~300 ml of 5% sucrose and 0.03% Silwet L-77 (Witco). This *Agrobacterium* suspension is

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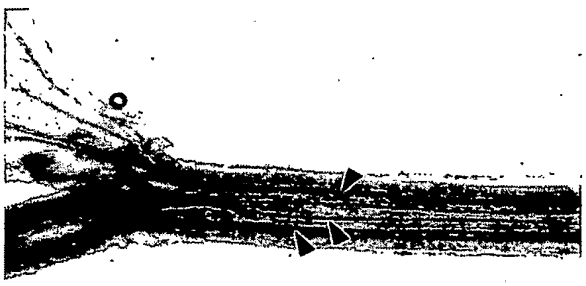
sprayed on the plants. The pots are placed in a tray which is covered with plastic wrap to maintain humidity. The plants are grown at 16/8 hours dark/light regime at 22 °C. Plants are grown to maturity to set seeds.

Seeds are harvested, surface sterilized in a solution containing 50% bleach and 0.02% Triton X-100 for 7 minutes. Seeds are then rinsed 3 times in sterile distilled water and plated out in MS medium containing 6 mg/l of Finale (Bayer) as selective agent. After 5 to 7 days, transformants are visible as green plants. Transformed plants are transferred onto new selection plates and after 6-10 days, are transferred to pots containing vermiculite and grown under conditions of 16 hours light/ 8 hours dark at 22 °C.

The inflorescence stem of the transformed plants are cut before flowering and histologically stained for GUS activity. Subsequent cuttings induces the formation of secondary xylem at the basis of plants that could also be histological stained for GUS activity.



A

B

In the plates above, activity of beta-glucuronidase in flowering stems of transgenic *Arabidopsis* plants is shown. These transgenic *Arabidopsis* plants were transformed with a construct containing the gene *Gus* operably linked to the cambium/xylem-preferred promoters of the invention, TUB (SEQ ID.: 2) (A) and C4H (SEQ ID.: 6) (B). Darker bands along the longitudinal axis of the stem (arrowheads) represent primary vascular bundles stained blue after the chromogenic assay, indicating the functionality and tissue-specificity of the respective promoter in each transgenic line.

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<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 3
<211> LENGTH: 2041
<212> TYPE: DNA
<213> ORGANISM: Populus sp.
<220> FEATURE:
<221> NAME/KEY: promoter
<222> LOCATION: (1)...(2041)
<223> OTHER INFORMATION: Arabinogalactan protein (ARAB) promoter

<400> SEQUENCE: 3

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<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 4
<211> LENGTH: 2422
<212> TYPE: DNA
<213> ORGANISM: Populus sp.
<220> FEATURE:
<221> NAME/KEY: promoter
<222> LOCATION: (1)...(2422)
<223> OTHER INFORMATION: Caffeic acid 3-O-methyltransferase (COMT) promoter

<400> SEQUENCE: 4

cataatatca aaacttaagc agatcaaatt gaaatatatt tgtaattttt atataaatta gcactgatat 70
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<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 5
<211> LENGTH: 793
<212> TYPE: DNA
<213> ORGANISM: Populus sp.
<220> FEATURE:
<221> NAME/KEY: promoter
<222> LOCATION: (1)...(793)
<223> OTHER INFORMATION: cinnamyl alcohol dehydrogenase (CAD) promoter

<400> SEQUENCE: 5

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cacagattca gtgttcttaa ctattatgtt ccctttttgtt tcccaagaac catgagttaa tcaggaccat 490
cgatactact gaggcccccac caatgttttg atcatgtgga caatgttcac ttgattttca actttgaaga 560
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tgtttcaaga aaatggtaga tct 793

<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 6
<211> LENGTH: 984
<212> TYPE: DNA
<213> ORGANISM: Populus sp.
<220> FEATURE:
<221> NAME/KEY: promoter
<222> LOCATION: (1)...(984)
<223> OTHER INFORMATION: cinnamate 4-hydroxylase (C4H) promoter

<400> SEQUENCE: 6

tgatatgaga aactaacgtt gcttgaattc aagatagaaa ttgaccttgc aagaagacaa acgtattctt 70
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<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 7
<211> LENGTH: 1007
<212> TYPE: DNA
<213> ORGANISM: Populus sp.
<220> FEATURE:
<221> NAME/KEY: promoter
<222> LOCATION: (1)...(1007)
<223> OTHER INFORMATION: cinnamoyl CoA reductase (CCR) promoter

<400> SEQUENCE: 7

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<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 8
<211> LENGTH: 2024
<212> TYPE: DNA
<213> ORGANISM: Populus sp.
<220> FEATURE:
<221> NAME/KEY: promoter
<222> LOCATION: (1)...(2024)
<223> OTHER INFORMATION: ferulate-5-hydroxylase (F5H) promoter

<400> SEQUENCE: 8

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aataatacaa tttagatccc tttaagcttt gcaaatttca ttttgatata aaagtttatt atatctctaa 140
ttaatataat tacaagtttg ccataatcaa aatttttttat ttttttattca atttcaatac tttaaacatt 210

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<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 9
<211> LENGTH: 995
<212> TYPE: DNA
<213> ORGANISM: Populus sp.
<220> FEATURE:
<221> NAME/KEY: promoter
<222> LOCATION: (1)...(995)
<223> OTHER INFORMATION: sinapyl alcohol dehydrogenase (SAD) promoter

<400> SEQUENCE: 9

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<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 10
<211> LENGTH: 1269
<212> TYPE: DNA
<213> ORGANISM: Populus sp.
<220> FEATURE:.
<221> NAME/KEY: promoter
<222> LOCATION: (1)...(1269)
<223> OTHER INFORMATION: UDP-D-glucuronate carboxy-lyase (UDP) promoter

<400> SEQUENCE: 10

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t 1331

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CLAIMS

What is claimed is:

1. An isolated nucleic acid molecule comprising a nucleotide sequence that is capable of initiating transcription of a gene in a plant cell, wherein said isolated nucleic acid molecule comprises: (i) a nucleotide sequence as set forth in SEQ ID NOS.: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12; (ii) 20 contiguous nucleotides of a nucleotide sequence set forth in SEQ ID NOS.: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12; or (iii) a nucleotide sequence that has at least about 65% sequence identity to a nucleotide sequence set forth in SEQ ID NOS.: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12.

2. An expression vector comprising: (i) the isolated nucleic acid molecule of claim 1, and (ii) a nucleic acid molecule which encodes a protein of interest, wherein (i) and (ii) are in operable linkage, wherein (i) does not normally regulate (ii).

3. The expression vector of claim 2, wherein said expression vector is a plasmid.

4. A recombinant host cell, wherein said recombinant host cell is transformed or transfected with the isolated nucleic acid molecule of claim 1.

5. A recombinant host cell, wherein said recombinant host cell is transformed or transfected with the isolated nucleic acid molecule of claim 2.

6. A recombinant host cell, wherein the isolated nucleic acid of claim 1 is stably incorporated in said recombinant host cell's genome.

7. A recombinant host cell, wherein the expression vector of claim 2 is stably incorporated in said recombinant host cell's genome.

8. A method of making a recombinant host cell, said method comprising transforming or transfecting a cell with the expression vector of claim 2.

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9. A method of making a protein, said method comprising transforming or transfecting a cell with the expression vector of claim 2, and culturing said cell under conditions favorable for the expression of said protein.

10. The method of claim 8, wherein said recombinant host cell is a plant cell.

11. A method for making a protein, said method comprising culturing a plant or plant part which comprises the recombinant host cell of claim 10, under conditions favoring production of said protein by said plant or plant part.

12. The method of claim 11, wherein said plant is a dicot.

13. The method of claim 12, wherein said dicot is *Eucalyptus*.

14. The method of claim 12, wherein said dicot is *Populus*.

15. The method of claim 11, wherein said plant is a monocot.

16. The method of claim 11, wherein said plant is a gymnosperm.

17. The method of claim 16, wherein said gymnosperm is *Pinus*.

18. The recombinant host cell of claim 4, wherein said recombinant host cell is a plant cell.

19. A plant comprising the recombinant plant cell of claim 18.

20. The plant of claim 19, wherein said plant is a dicot.

21. The plant of claim 20, wherein said dicot is *Eucalyptus*.

22. The plant of claim 20, wherein said dicot is *Populus*.

23. The plant of claim 19, wherein said plant is a monocot.

24. The plant of claim 19, wherein said plant is a gymnosperm.

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25. The plant of claim 24, wherein said gymnosperm is *Pinus*.

26. A seed comprising the recombinant plant cell of claim 18.

27. The recombinant host cell of claim 4, wherein said recombinant host cell is a pollen cell.

28. The method of claim 11, wherein said plant part is selected from the group consisting of a root, stem, leaf, flower, fruit, seed, pistil, stigma, style, ovary, ovule, stamen, anther, and filament.

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TITLE: CAMBIUM/XYLEM-PREFERRED PROMOTERS AND USES THEREOF

Inventor(s): Fabio Papes
Isabel Rodrigues Gerhardt
Paulo Arruda

ABSTRACT

The present invention relates to nucleic acid molecules corresponding to regulatory portions of genes whose expression is predominant in cambium and/or xylem. The invention also relates to compositions and methods of using the same to regulate the expression, in a cambium/xylem-preferred manner, of genes and/or any kind of nucleotide sequences in a plant. Nucleic acid molecules and its compositions include novel nucleotide sequences for cambium/xylem-preferred promoters identified and isolated from populus (*Populus spp*) genes encoding sucrose synthase (SuSy), alpha-tubulin (TUB), arabinogalactan protein (ARAB), caffeic acid 3-O-methyltransferase (COMT), cinnamyl alcohol dehydrogenase (CAD), cinnamate 4-hydroxylase (C4H), cinnamoyl CoA reductase (CCR), ferulate-5-hydroxylase (F5H), sinapyl alcohol dehydrogenase (SAD), UDP-D-glucuronate carboxy-lyase (UDP), lipid transfer protein (LTP) and ag-13 (AG13). Methods for expressing genes and/or any kind of nucleotide sequences in a plant using the promoter sequences disclosed herein are provided. The methods comprise stably incorporating into the genome of a plant cell a nucleotide sequence operably linked to a cambium/xylem-preferred promoter of the present invention and regenerating a stably transformed plant that expresses the nucleotide sequence.

21 Claims, 16 Drawing Sheets

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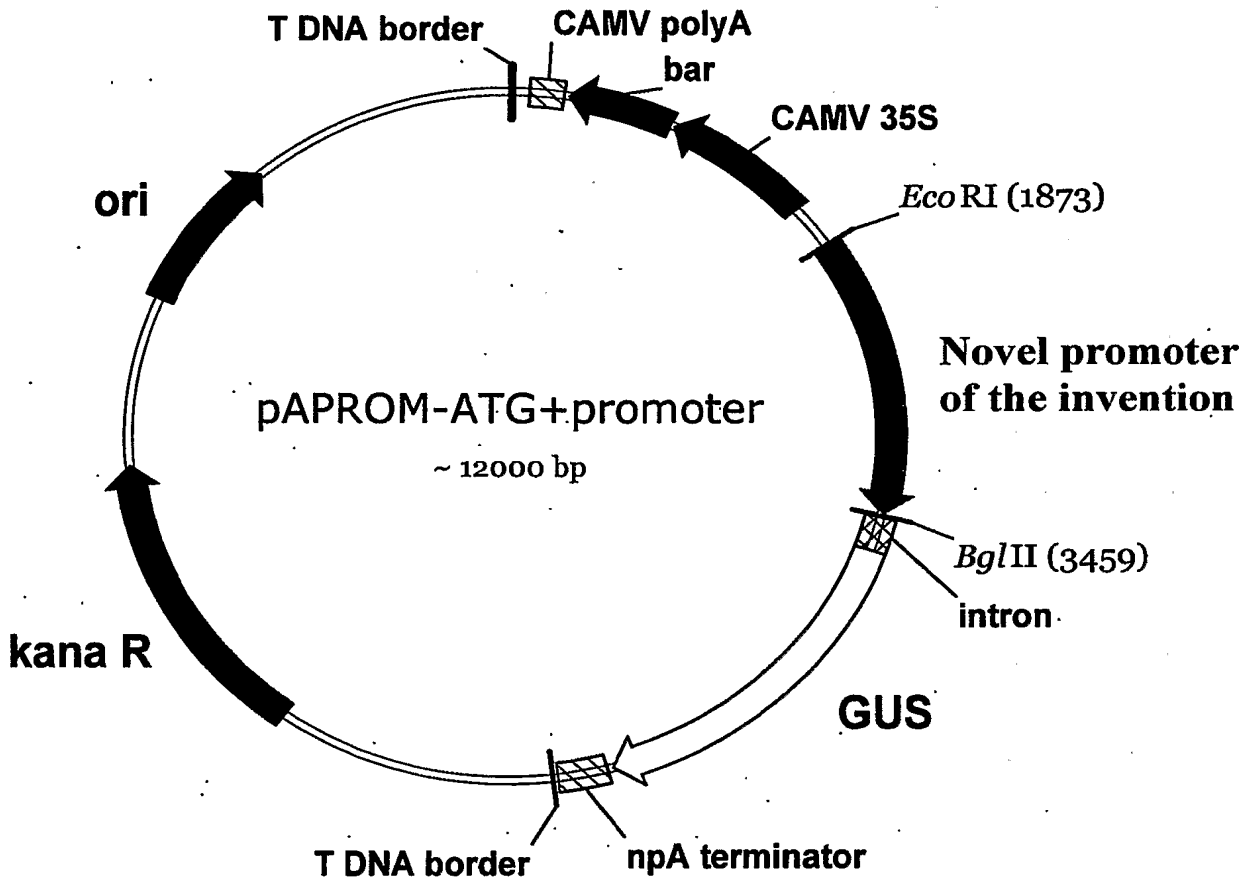
FIG. 1.



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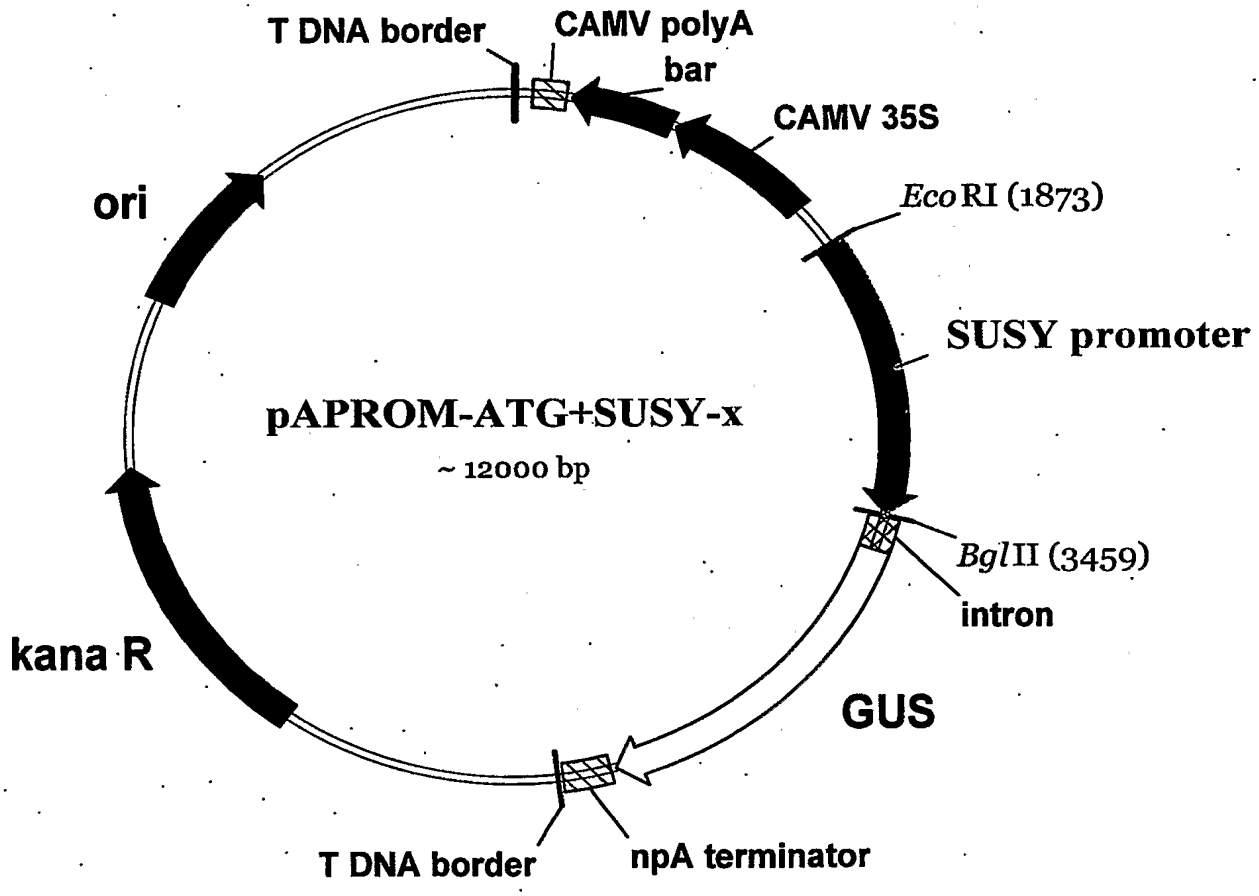

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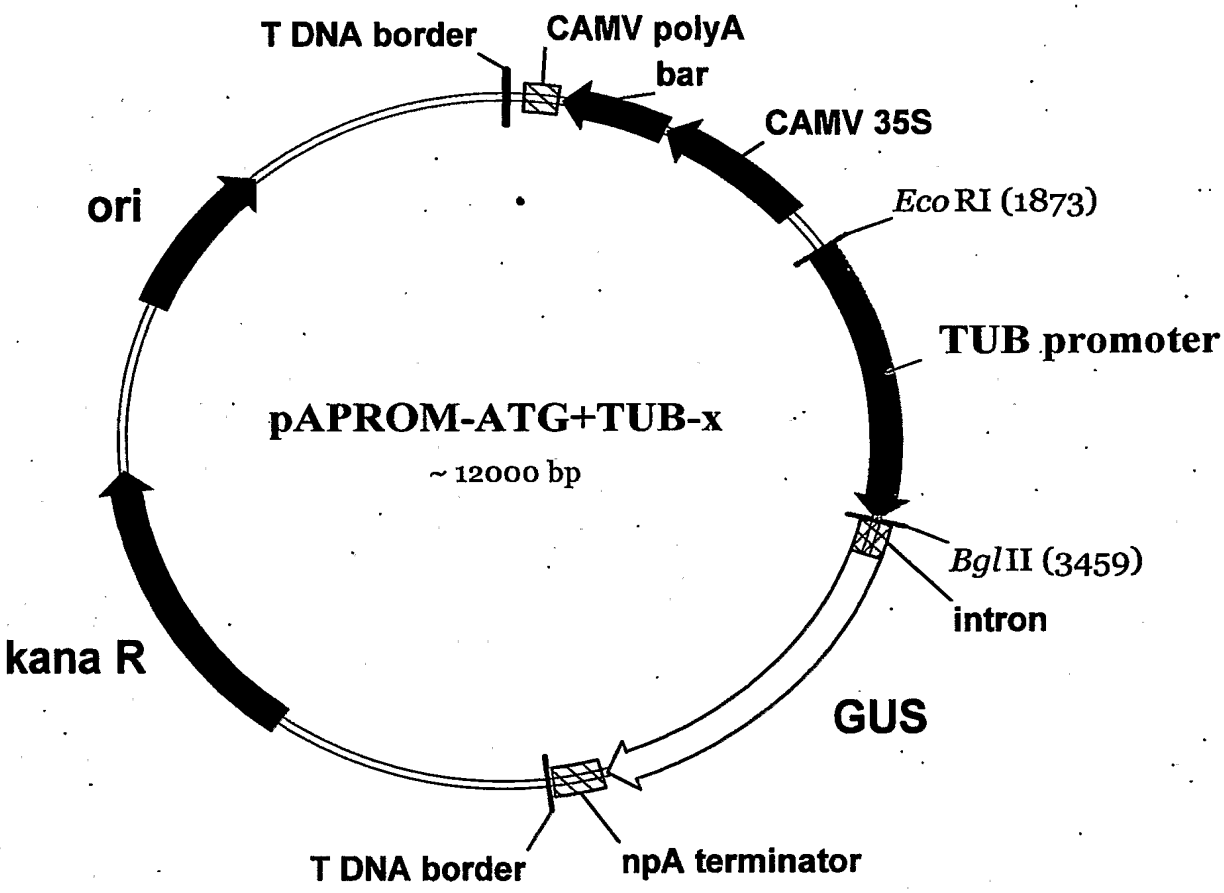

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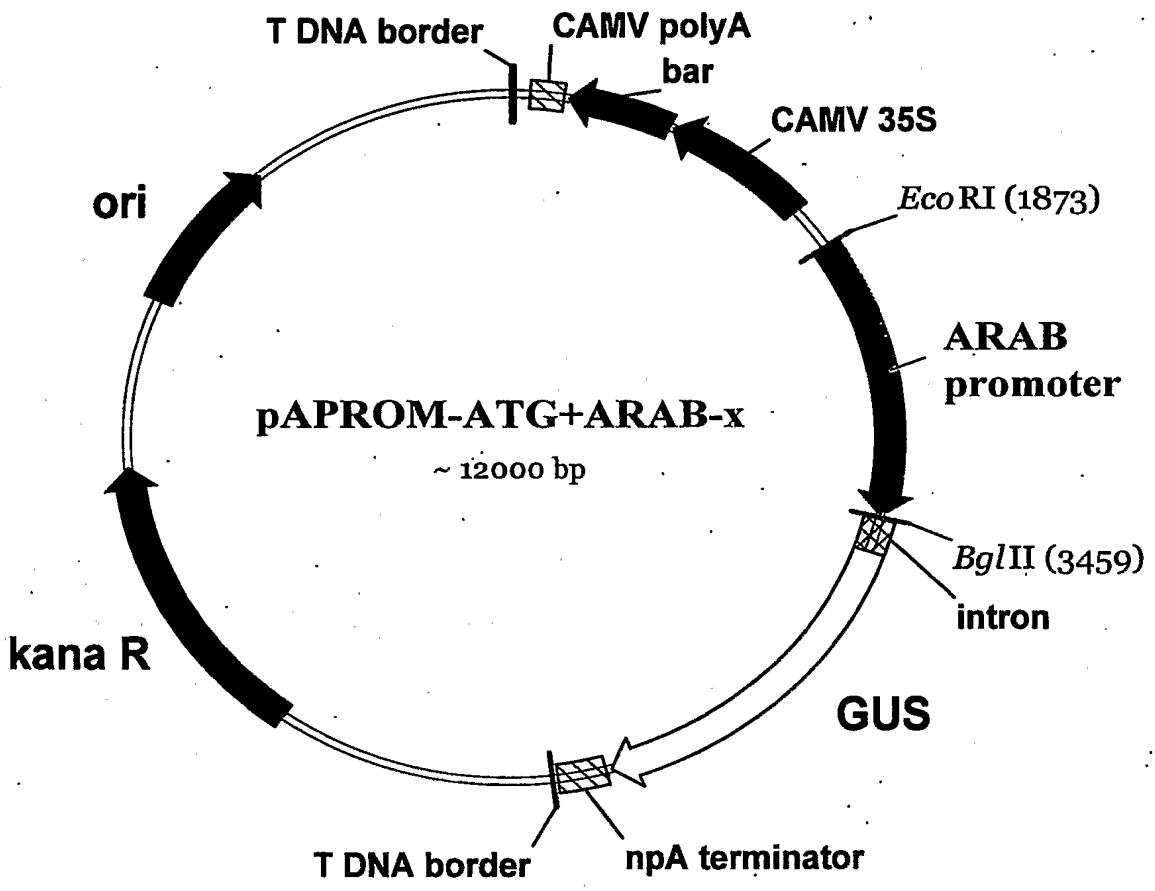

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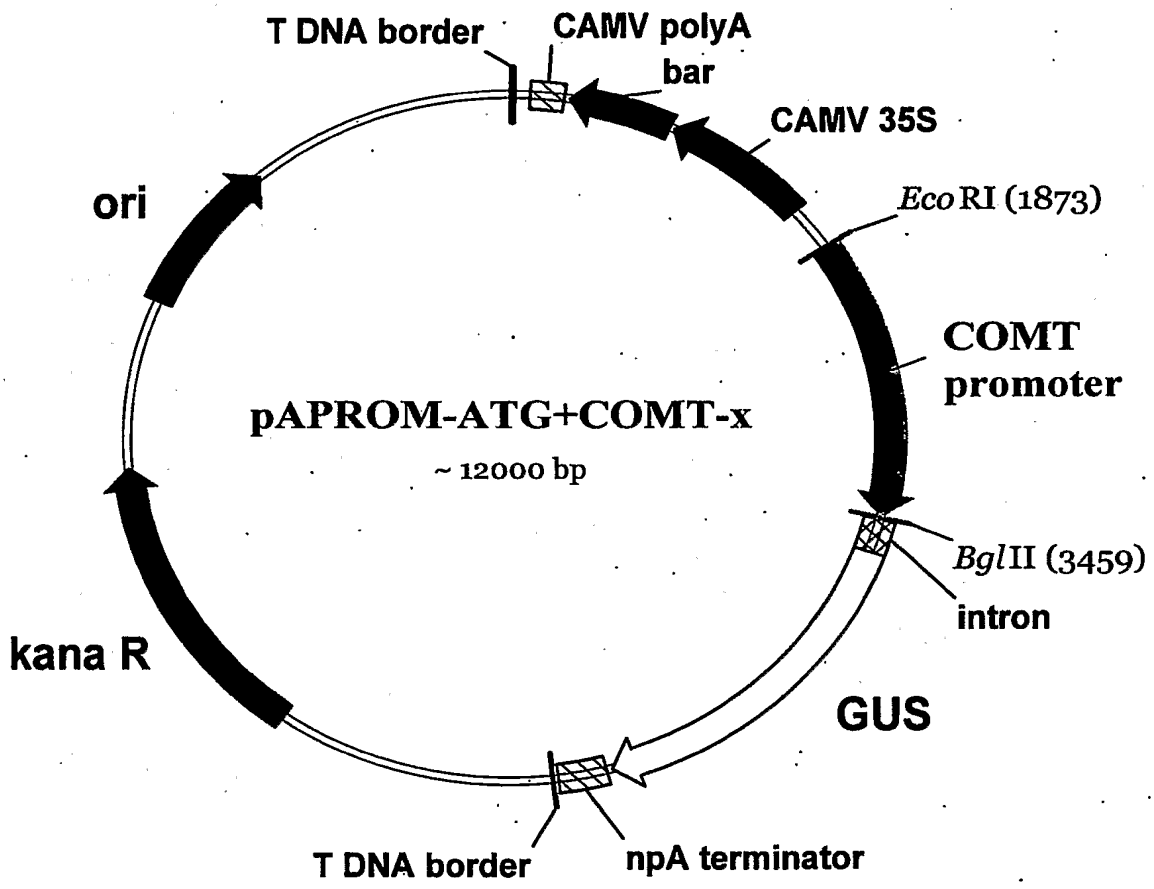

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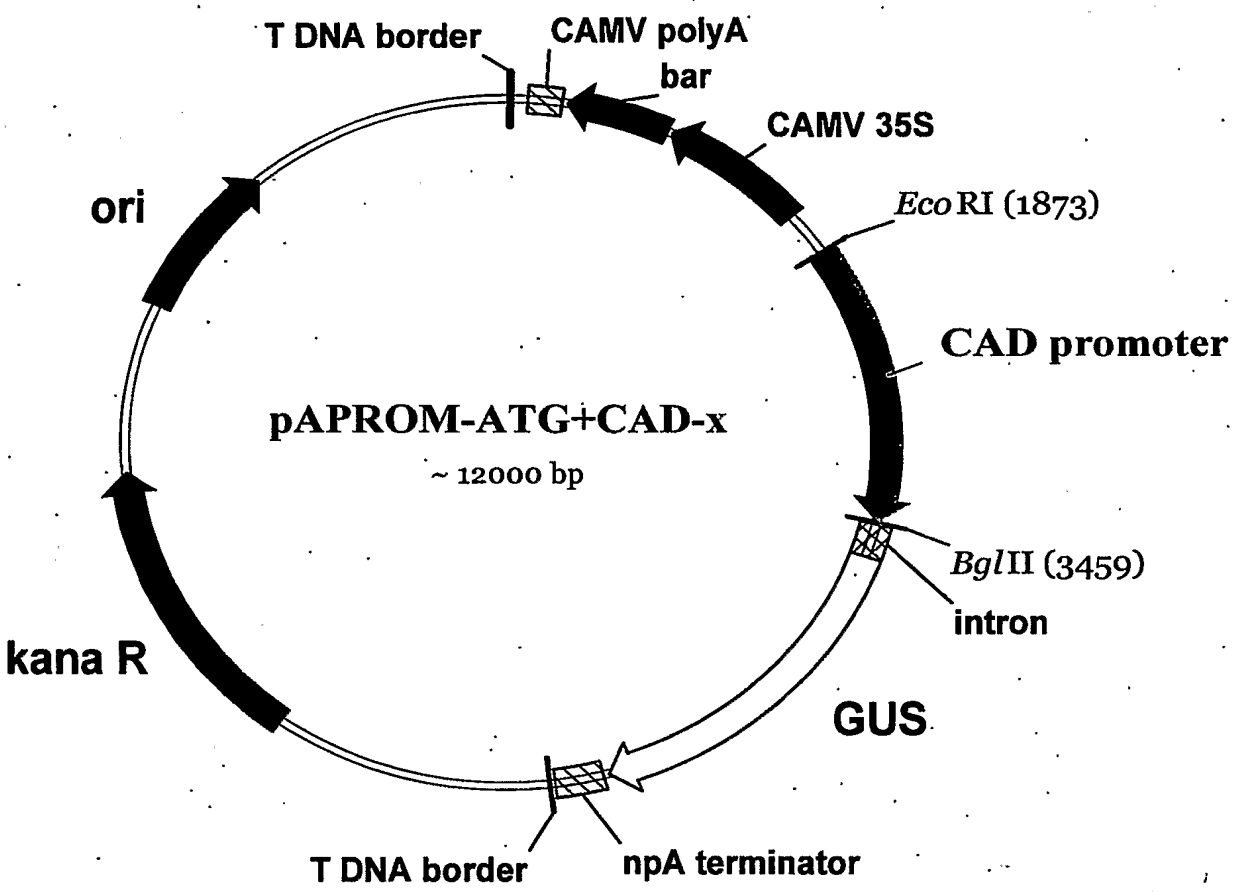

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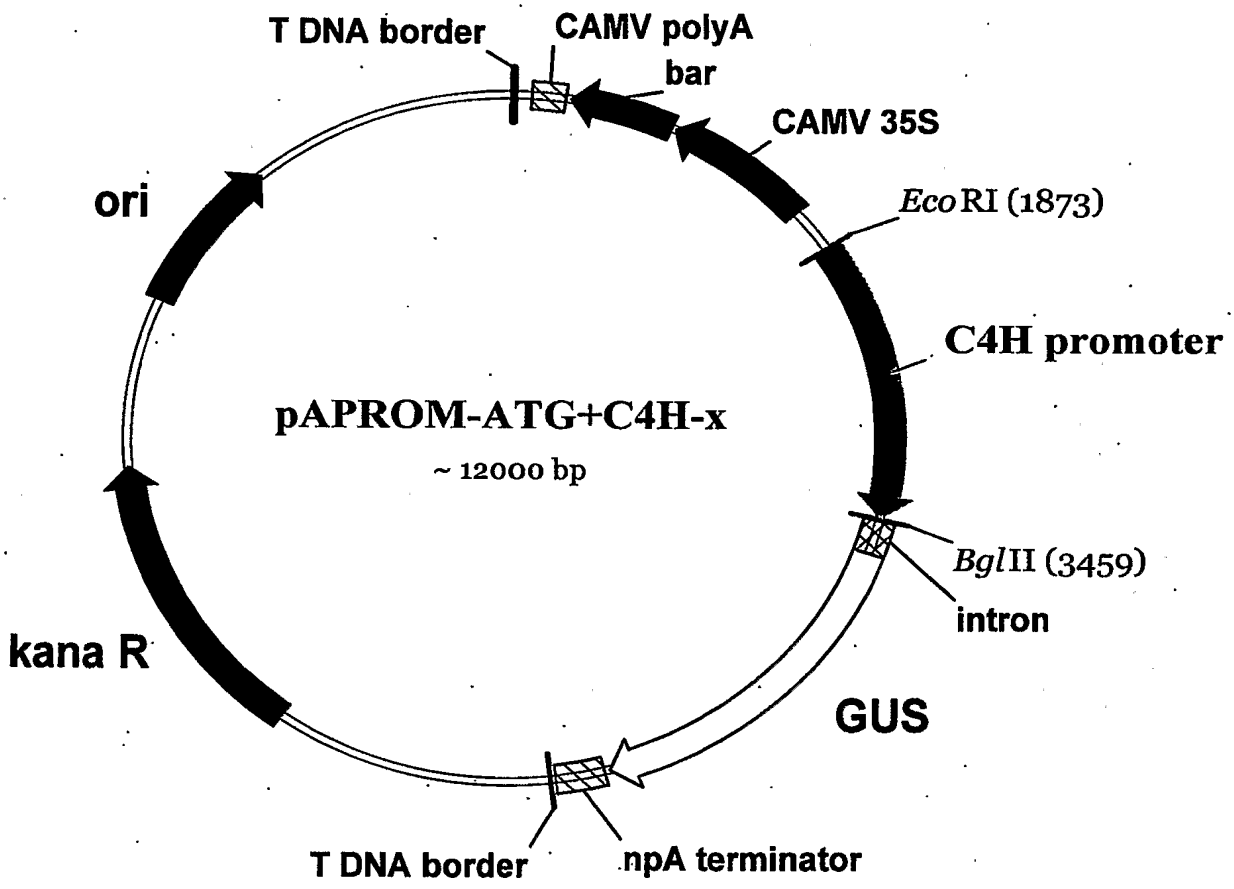

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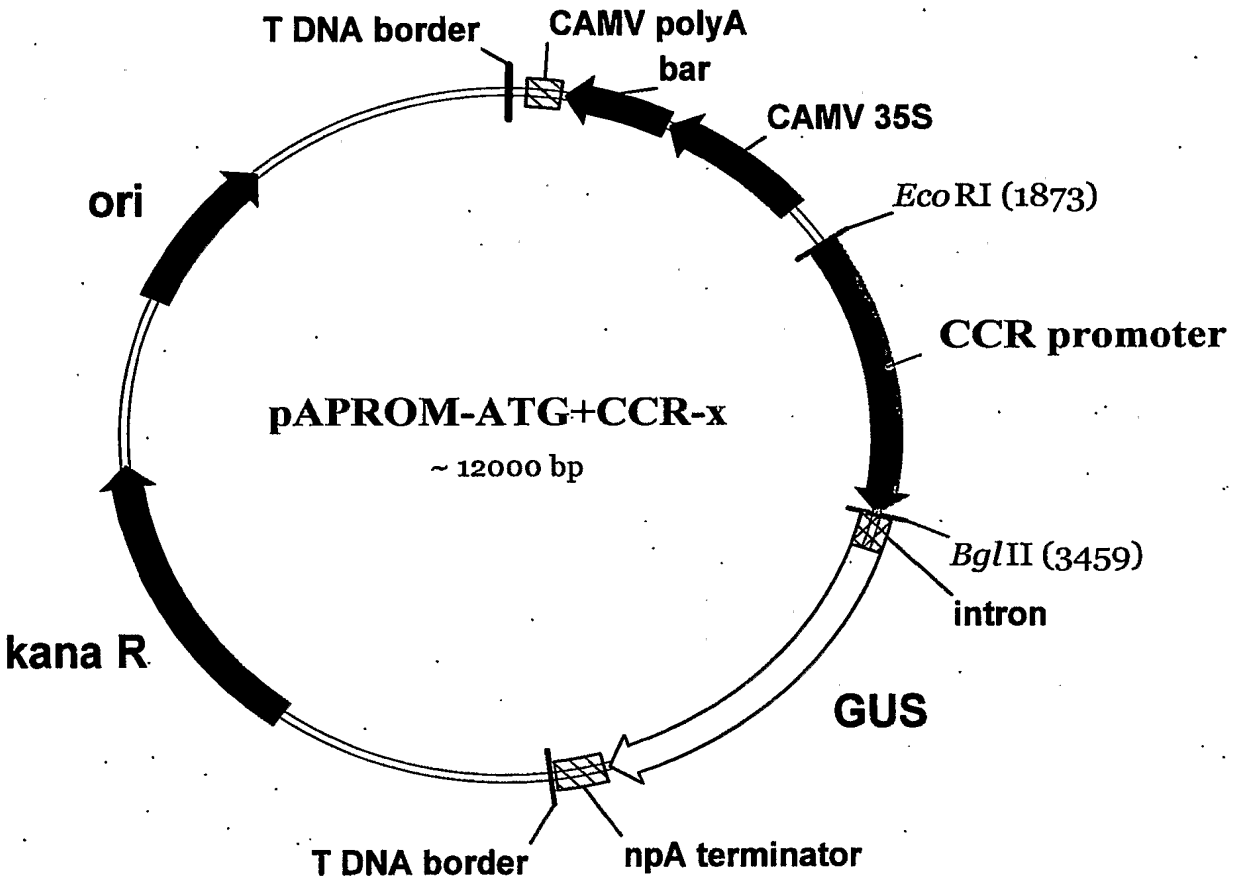

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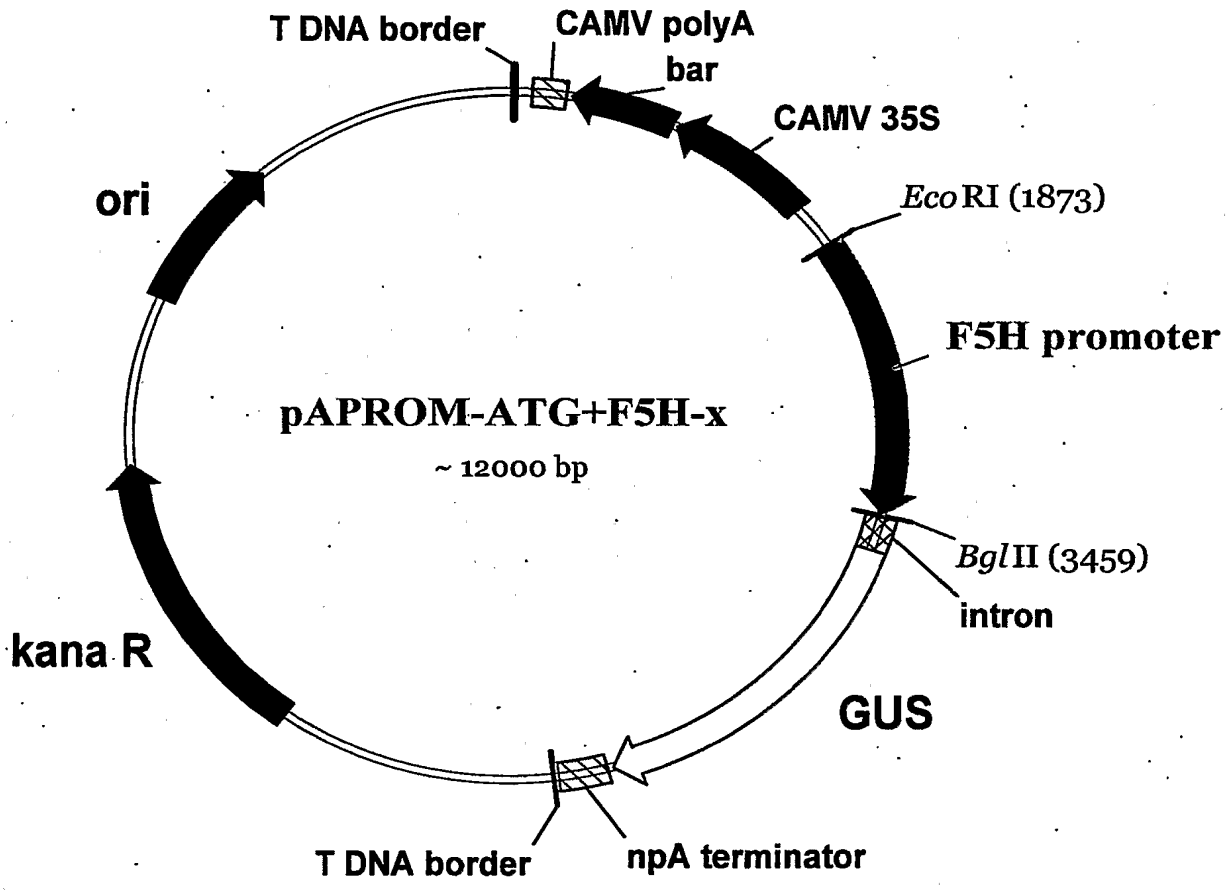

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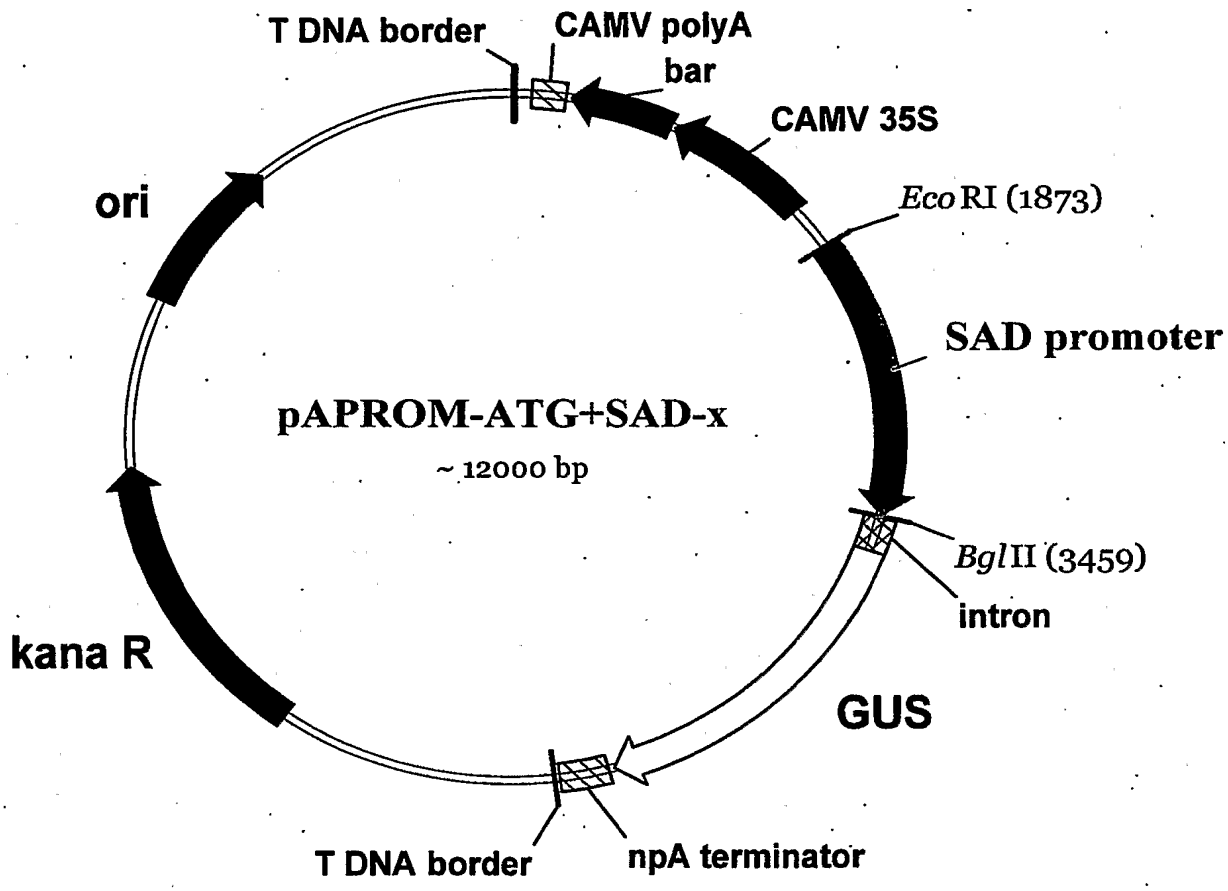

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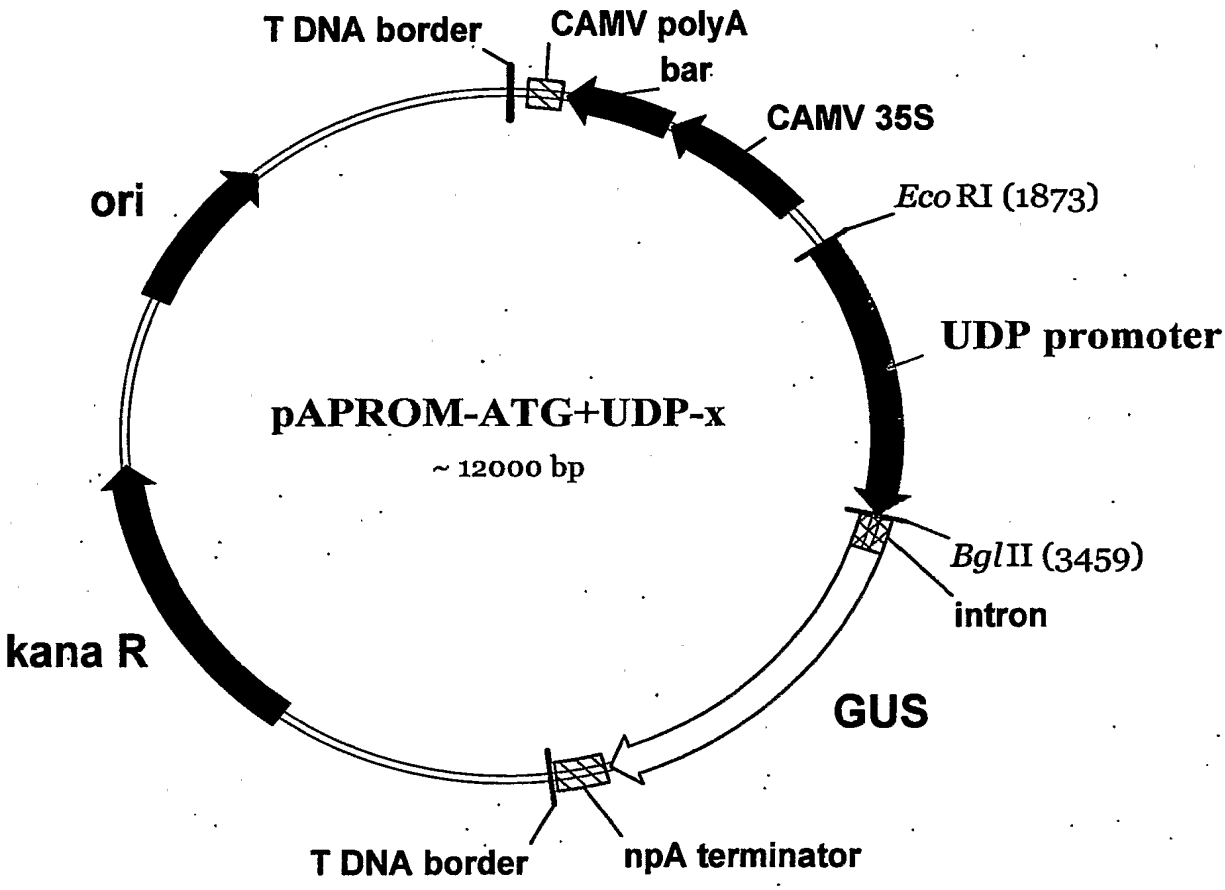

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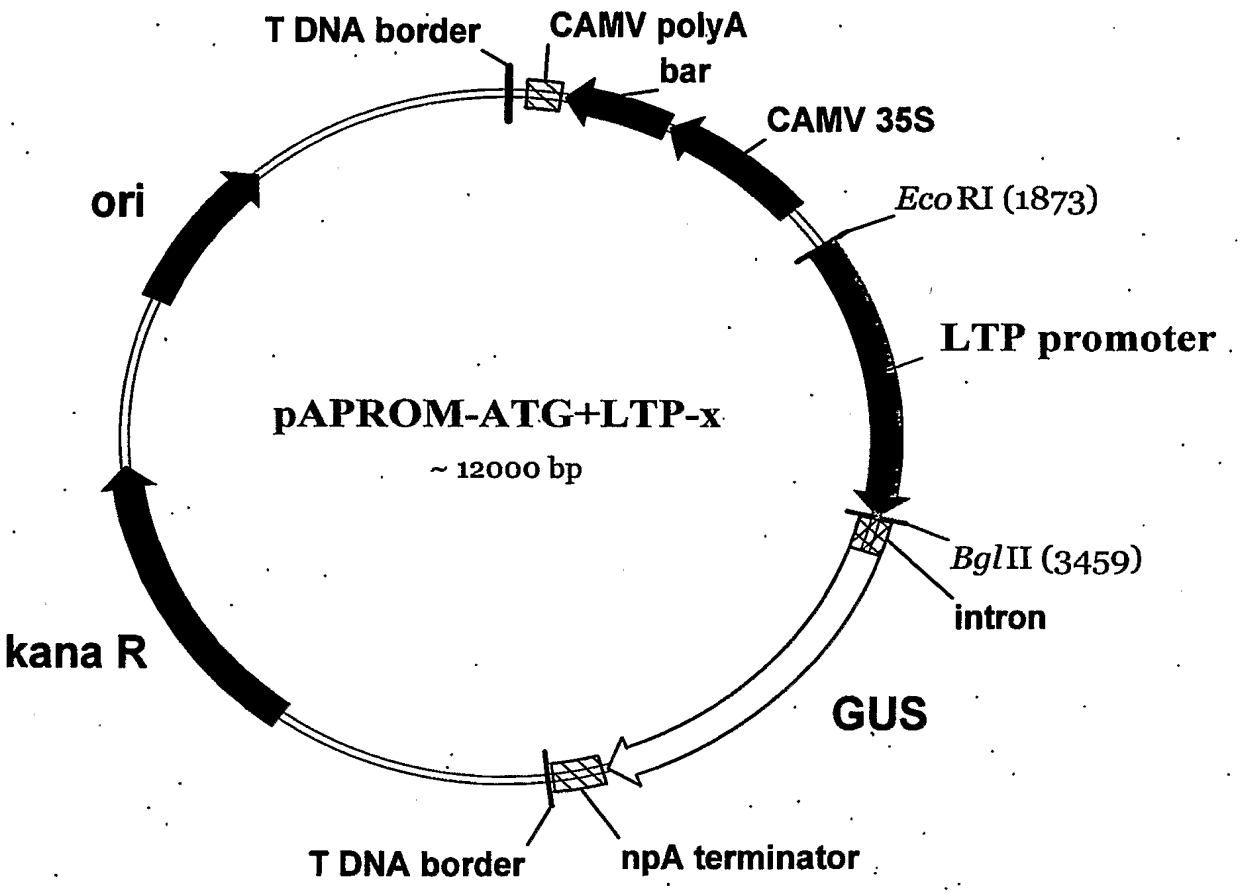

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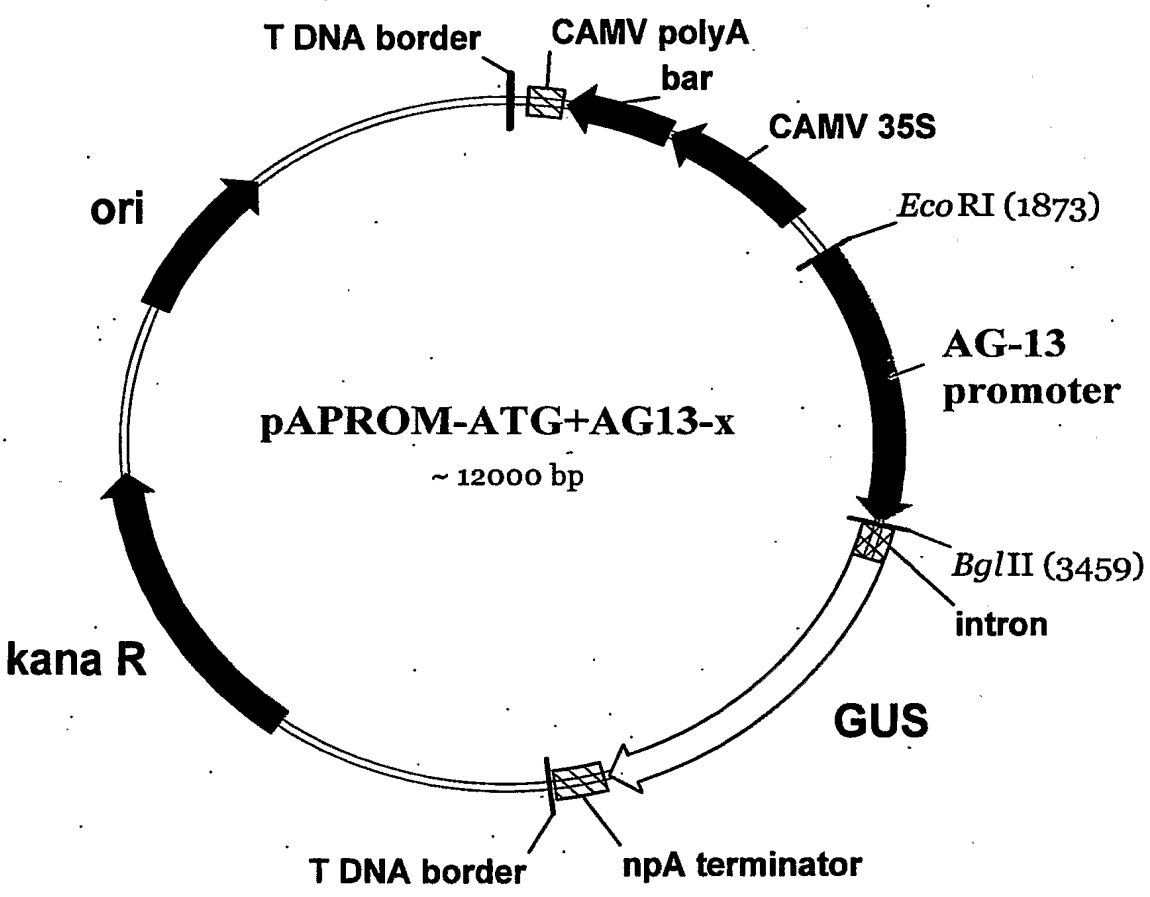

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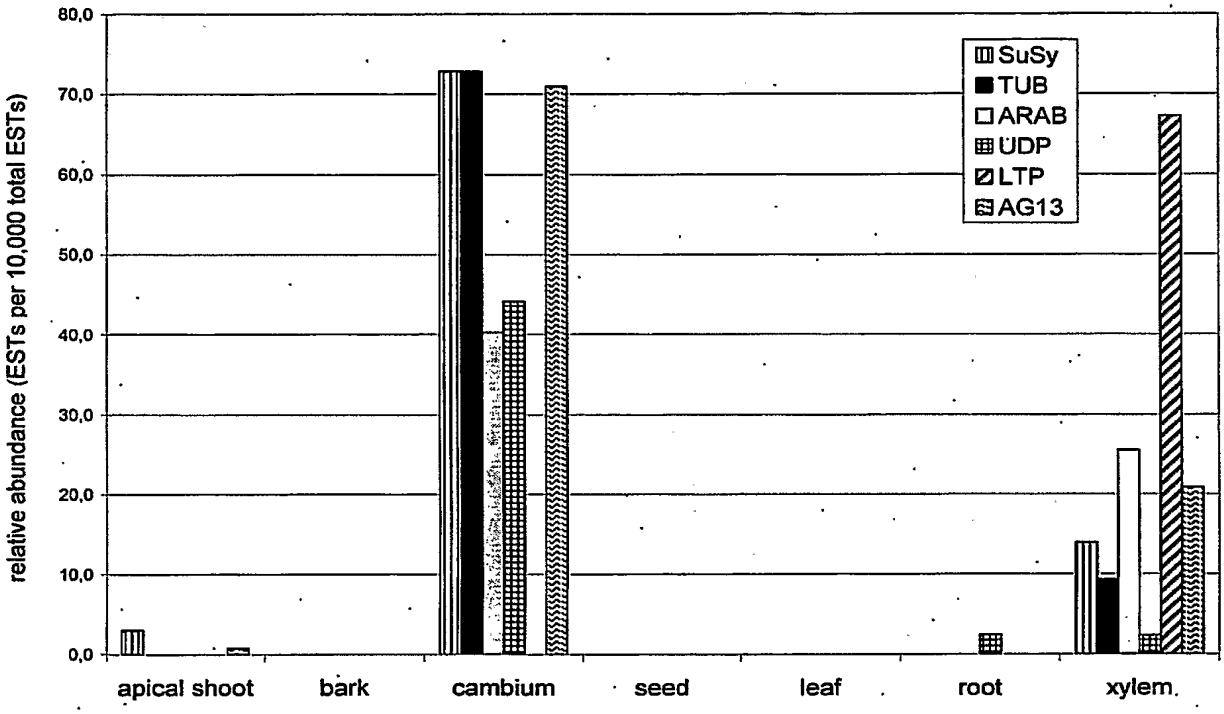

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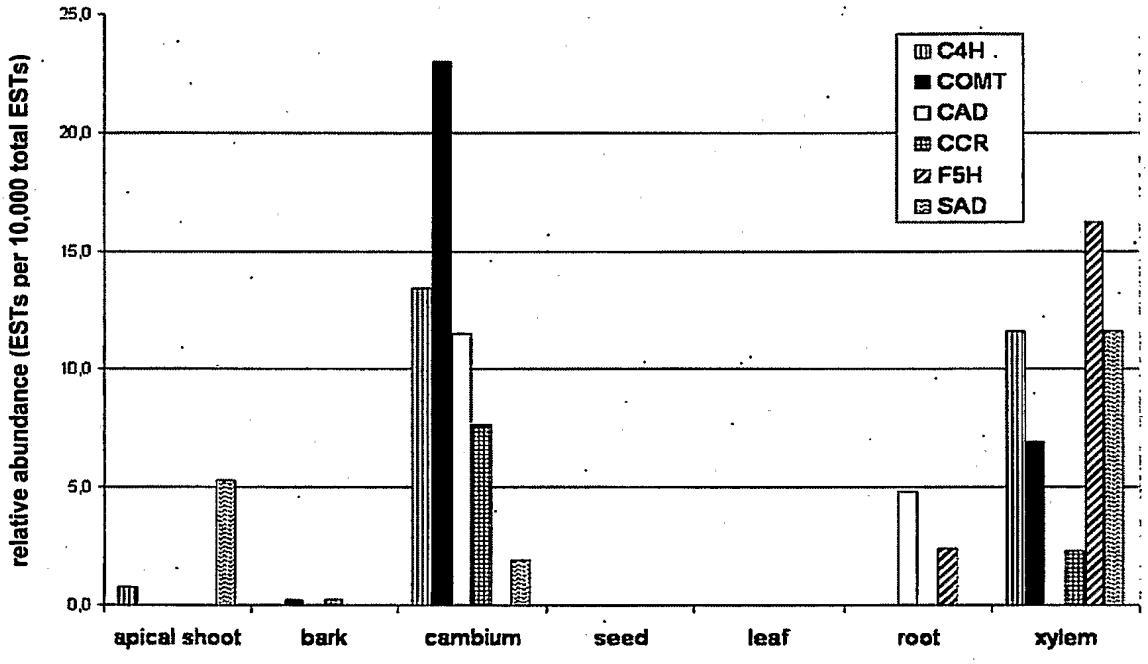

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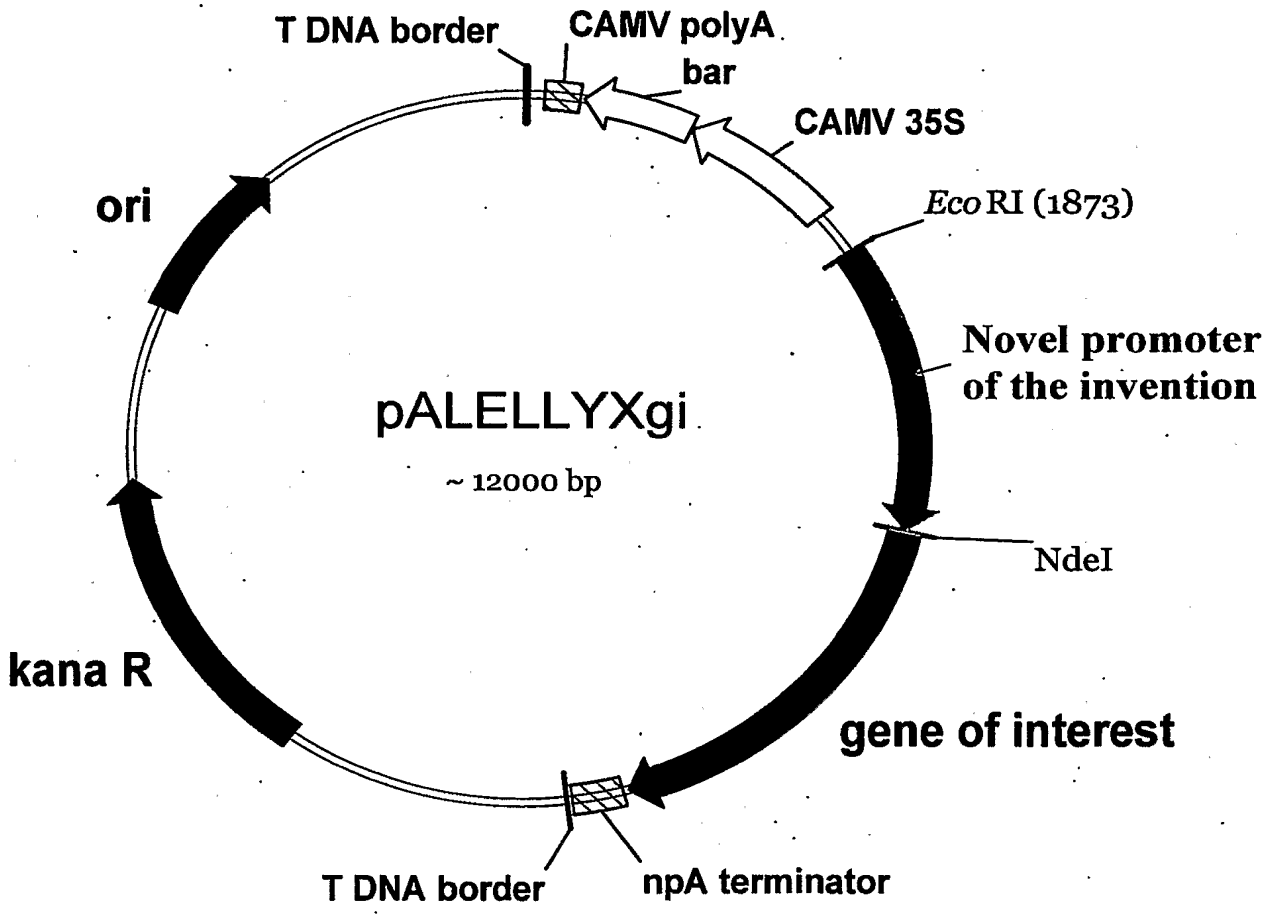

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